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Antioxidant activities of *Zizyphus mauritiana* Lam. (Rhamnaceae)

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ABSTRACT : In the present study, antioxidant activities of the two varieties of fruits of *Zizyphus mauritiana* (Boroi) were investigated. The IC_{50}'s of the ethanolic extracts of *Zizyphus mauritiana* and *Zizyphus mauritiana* (Narikeli) were 72 and 250 µg/ml respectively. Among these two varieties local variety *Zizyphus mauritiana* (Beri) showed higher antioxidant activities. Therefore, local variety of *Zizyphus mauritiana* found beneficial for human health.

Keywords : Antioxidant activity, *Zizyphus mauritiana*, Ethanol extracts, DPPH free radical, IC_{50}

INTRODUCTION

Antioxidant means "against oxidation." An antioxidant is any substance that retards or prevents deterioration, damage or destruction by oxidation (Dekkers *et al.*, 1996). It is a classification of several organic substances, including vitamins C and E, vitamin A (which is converted from beta-carotene), selenium and a group known as the carotenoids (Kaczmarski *et al.*, 1999; Dekkers *et al.*, 1996).

A free radical is a compound with one or more unpaired electrons in its outer orbital (Jesberger and Richardson 1991). Such unpaired electrons make these species very unstable and therefore quite reactive with other molecules due to the presence of unpaired electron(s) (Karlsson 1997) and they try to pair their electron(s) and generate a more stable compound. The most dangerous free radicals are the atomic and molecular varieties of oxygen which is known as Reactive Oxygen Species (ROS). While ROS are not technically free radicals, they are highly reactive with the molecules around them (Sharma and Clark 1998). ROS is a collective term, which includes not only the oxygen radicals ($Ö_2$, and $^{\cdot}OH$) but also some non-radical derivatives of oxygen, including hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3) (Sjodin *et al.*, 1990).

The human body is composed of many different types of cells. Cells are composed of many different types of molecules. Molecules consist of one or more atoms of one or more elements joined by chemical bonds. Normally, bonds don't split in a way that leaves a molecule with an odd, unpaired electron (Halliwell, 1989). But when weak bonds split, free radicals are formed (Karlsson 1997). Free radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. Generally, free radicals attack the nearest stable molecule, "stealing" its electron. When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction and can be "thousand of events long" (Goldfarb 1999). Once the process is started, it can cascade, finally resulting in the disruption of a living cell (Jesberger and Richardson 1991).

Free radicals are formed continuously as normal by-products of oxygen metabolism during mitochondrial oxidative phosphorylation. Thus the mitochondrion is the main source of free radicals (Przedborski and Jackson-Lewwis 1998, Fahn and Cohen 1992).

The role of free radicals in many disease conditions has been well established. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging crucial bio-molecules. If they are not effectively scavenged by cellular constituents, they lead to disease conditions (Halliwell, 1994; Halliwell and Gutteridge 1985) e.g. Cerebrovascular Disease, Cancer, Arteriosclerosis, Atherosclerosis, Heart Disease, Senility, Aging, Behcet's Disease, Crohn's Disease, Cataracts, Sunburn, Ulcers, Osteoporosis, Rheumatoid Arthritis, Diabetes Mellitus, Emphysema, Stroke (Sharma and Clark 1998), Rheumatoid Arthritis, Hemorrhagic Shock, Cardiovascular Disorders, Cystic Fibrosis, Neurodegenerative Diseases (e.g. Parkinsonism, Alzheimer's disease), Gastrointestinal Ulcerogenesis, AIDS and even early Senescence (Halliwell, 1994; Halliwell and Gutteridge). Free radicals also spoil foods and degrade materials such as rubber, gasoline and lubricating oils (Dexter *et al.*, 1994). Antioxidants can take the form of enzymes in the body, vitamin supplements, or industrial additives. They are routinely added to metals, oils, foodstuffs, and other materials to prevent free radical damage. To neutralize these free radicals antioxidant plays an important role (Vani *et al.*, 1997).

In recent years, one of the areas which attracted a great deal of attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. Several plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity (Larson, 1988; Tripathi 1996; Vani *et al*., 1997). In the present study, we investigated the antioxidant activity of the crude extracts of the two varieties of *Zizyphus mauritiana.*

MATERIALS AND METHODS

Measurement of Antioxidant Activities

The antioxidant activity of the ethanol extracts was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. 1ml of each solution of different concentrations (1-500µg/ml) of the extracts was added to 3 ml of 0.004% ethanol DPPH free radical solution. After 30 minutes the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid concentrations (1-500 µg/ml).The method described by Hatano *et al.* (1988) was used to measure the absorbance with some modifications. Then the % inhibition was calculated by the following equation:

% radical scavenging activity = (absorbance of blank-absorbance of sample)/(absorbance of blank) x 100

From calibration curves, obtained from different concentrations of the extracts, the IC_{50} (Inhibitory concentration 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals (Gupta *et al.*, 2003).

Procedure

Table 1 : Showing detailed data on extraction of fruits.

1. At first, 6 test tubes were taken to make aliquots of 6 conc. (1, 5, 10, 50, 100 and 500ìg/ml) with the samples.
2. Fruit extracts and ascorbic acid were weighed accurately and dissolved in ethanol to make the required concentrations by dilution technique. Here ascorbic acid was taken as standard.
3. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously magnetic stirrer was used.
4. After making the desired concentrations 3ml of 0.004% DPPH solution was applied on each test tube by pipette.
5. The room temperature was recorded and kept the test tubes for 30 mins in light to complete the reactions.
6. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank.
7. After 30 minutes, the absorbance of each test tube was taken by a UV spectrophotometer.
8. IC_{50}'s were measured from % Inhibition *vs.* Conc. graphs.

RESULTS AND DISCUSSION

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study, ethanolic extracts of the two varieties of the fruit of *Zizyphus mauritiana* (Boroi) showed potential free-radical scavenging activity. The antioxidant activities of the individual compounds, present in the extracts may depend on structural factors, such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features (Patt *et al.*, 1990).

From the graph 1 and 2, it is found that IC_{50} of the fruit extract of *Zizyphus mauritiana* is 72ìg/ml which indicates the remarkable antioxidant activity of the extract. The antioxidant activity is to the presence of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features (Patt *et al.*, 1990).

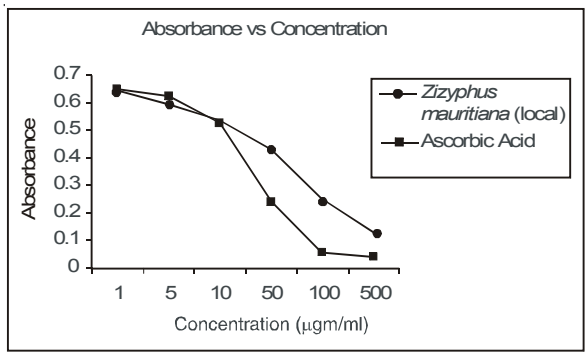

Fig.1. DPPH scavenging Assay of the fruit extract of *Zizyphus mauritiana* compared with standard ascorbic acid.

Table 1 : The detailed data on extraction of two fruit varieties.

Name of fruit	Type	Wt. during collection (gm)	Wt. after drying (g)	% of water in green fruits	Wt. used for extraction (g)	Etanol used (ml)	Wt. of bottles used for evaporation (g)	Wt. of bottles with extract after evaporation (g)	Wt. of the final extract (g)
Z. mauritiana	Mature	1000	115	88.50	50	150	48.50	54.50	6
Z. mauritiana (narkikeli)	Mature	1000	118	88.20	50	150	57.50	63.00	5.5

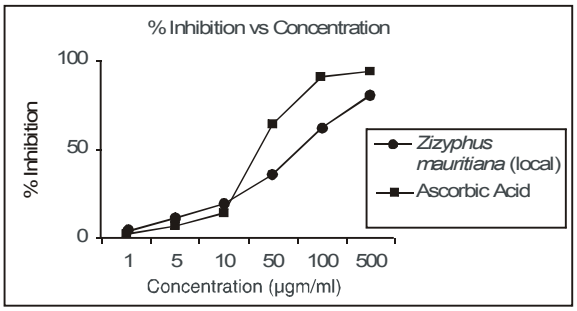

Fig.2. Evaluation of IC_{50} of the fruit extract of *Zizyphus mauritiana* and standard ascorbic acid.

From the graph 3 and 4, it is found that IC_{50} of the fruit extract of *Zizyphus mauritiana* (Narikeli) 250ìg/ml which indicates that low level of antioxidant activity with compare to local variety. The antioxidant activity is due to the presence of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features (Patt and Hudson 1990).

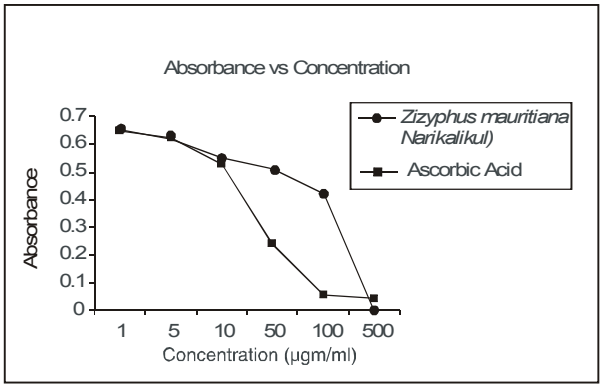

Fig.3. DPPH scavenging assay of the fruit extract of *Zizyphus mauritiana* (Narikeli) compared with standard ascorbic acid.

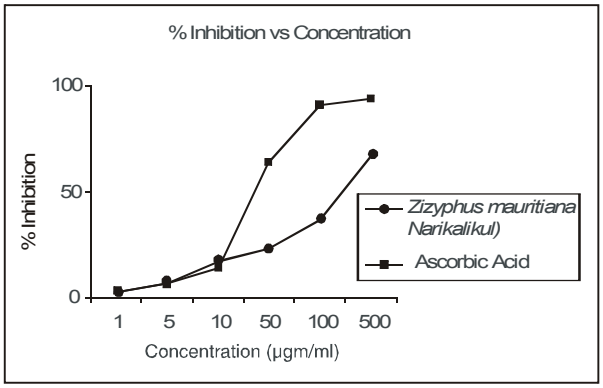

Fig.4. Evaluation of IC_{50} of the fruit extract of *Zizyphus mauritiana* (Narikeli) and standard ascorbic acid.

In the present study, the extracts were prepared using 99.99% ethanol and 6.0 and 5.5 gm final extracts were collected from 50 gm fruit powder of each two varieties of *Zizyphus mauritian*a. These results indicate that the extraction with ethanol gives a considerable amount of extracts.

The *in vitro* antioxidant activities of the prepared fruit extracts were investigated by DPPH free radical scavenging assay. The model system of scavenging DPPH free radicals is a simple and acceptable method to evaluate the antioxidative activity of antioxidants. It is accepted that the DPPH free radical scavenging by antioxidants is due to their hydrogen donating ability (Chen and Ho 1995). The collected fruit extracts exhibited remarkable DPPH free radicals scavenging ability at different concentrations. From these, the % inhibition concentrations and IC_{50}'s were calculated. The measured IC_{50} 0f *Zizyphus mauritiana* was 72µg/ml and *Zizyphus mauritiana* (Narikeli) was 250µg/ml, this result indicates that the antioxidant activity of local variety is greater than the Narikeli variety.

The water extract of carob pods reduce 13% DPPH free radicals at a concentration of 53µg/ml (Shigenori *et al.*, 2002) whereas the ethanolic extract of the pods of *A. marmelos* showed the same effect at 49.4µg/ml. In case of grape pomace, its methanolic extract exhibits 73.65% hydroxyl radical scavenging activity at 200µg/ml (Kotamballi *et al.*, 2002). On the other hand, 0.2mg methanolic extract of oil seeds shows 50% inhibition of DPPH free radicals (Matthaus, 2002).

This experiments and also the observations of other groups e.g. Kotamballi *et al.* (2002), Matthaus (2002) supported that the extraction with 99.99% ethanol gives not only a considerable yield of extracts but also a high antioxidant activity. The antioxidant activities of the individual compounds may depend on structural factors, such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features (Patt and Hudson 1990).

Kaczmarski *et al.* (1999) reported that among the antioxidative compounds (vitamin C, E, A, selenium and carotenoids), ascorbic acid (Vitamin C) shows very strong intensity of antioxidative activities (Kaczmarski *et al.*, 1999).

CONCLUSION

India is a developing country and 27% people are below poverty line. It is impossible for them to take expensive antioxidative drugs or to eat the costly fruits like apple, grape fruits, orange, litchi, mango, pineapple and so on. In the present study, cheap local fruits of India are used that can easily be taken by the poor people. The investigation showed that the fat free residues of *Zizyphus mauritiana* whose used parts are edible contain considerable amount of antioxidative compounds because the extracts of these fruit has remarkable antioxidant activities. So, these fruits can be used by the poor people as biological antioxidants. But the other variety of *Zizyphus mauritiana* name Narikeli show low level of antioxidant.

The investigation showed only the intensity of antioxidative compounds present in the extracts. But the study gave no concrete indication of the names of the antioxidative compounds or exact amounts of them. More work should be done to characterize individual antioxidative compounds of these fruits in order to assign certain fruit(s) for using as the cheapest source of antioxidant to the poor people of India as well as the world. On the other hand, in most food industries, synthetic antioxidants e.g. butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), propyl gallate (PG) etc. are used in order to prevent the rancidity of processed foods. This experiment supports that these fruits can be used in such industries as natural antioxidants subjected to proper investigations.

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